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HISTOCHEMICAL CHANGES IN GALACTOSIDASE AND GLUCURONIDASE IN THE RETICULOENDOTHELIAL SYSTEM OF THE NEONATAL RAT

John R. Esterly
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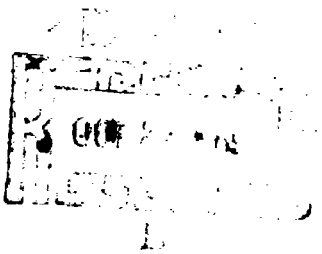

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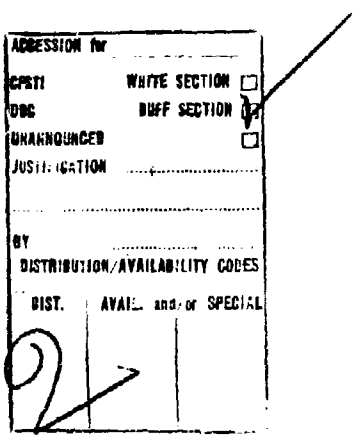

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In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

Changes in the histochemical reactivity and localization of β-D-galactosidase and β-D-glucuronidase were correlated with the morphologic differentiation of lymphoid tissues in neonatal rats. The thymus had a relatively mature histologic structure by 7 to 10 days of age, and maturation in the neonatal spleen and intestinal lymphoid tissue followed in that order. Differentiation in peripheral lymph nodes was noted between 1 and 6 months of age. The pattern of galactosidase reactivity in the thymus was delayed but roughly paralleled the histologic development. After an initial peak, glucuronidase staining was similar. In contrast, reactivity for both enzymes in the intestinal mononuclear cells anticipated the morphologic changes. In the spleen and peripheral lymph nodes, increases in both enzymes paralleled histologic maturation. These findings indicate that morphologic appearance may be a useful index of the activity of these lysosomal enzymes in developing lymphoid tissues. The minor discrepancies in enzymatic and morphologic maturation in the thymus and intestine suggest that changes in reactivity for these enzymes may be related to the proximity of antigenic stimulation.

I. INTRODUCTION*

In contrast to the majority of organs and tissues, in which morphogenesis is relatively complete at birth, the components of the reticuloendothelial system (RES) continue to develop and differentiate during the neonatal period and early infancy. Under normal conditions, functional maturation, such as humoral and cell-mediated immunity, is also incomplete. Phagocytic activity is absent or decreased in fetal and neonatal animals,[1-3] and mice can be rendered immunocompetent in the neonatal period by transfusion with adult macrophages.[4] For these reasons, changes in the activity of enzymes associated with macrophages and their lysosomal function[5] are of particular interest. In addition, the activity of these enzymes in the spleen increases after the injection of bovine serum albumin.[6] Halogen-substituted indolyl substrates for several hydrolytic enzymes have proved useful in the histochemical examination of lymphoid tissue because of their specificity, unusual resolution, and stability.[7,8] The present report describes the development of the RES in the rat and the application of methods for detection of β-D-galactosidase (GAL) and β-D-glucuronidase (GLCR) in neonatal reticuloendothelial tissues. The changes in enzyme reactivity are correlated with the degree of their morphologic development.

II. MATERIALS AND METHODS

The tissues used in this study were obtained from groups of four to six inbred Fischer 344 rats at each of the following ages: 19 and 21 day's gestation; newborn; 4, 7, 10, 14, and 28 days; and 6 months. The thymus, spleen, liver, lung, portions of proximal jejunum, distal ileum, and, when identifiable, mesenteric, axillary, and popliteal lymph nodes were removed from sacrificed animals and quickly frozen in a dry ice - acetone bath and stored at -70 C. Frozen sections were subsequently prepared, incubated in the halogen-substituted indolyl galactoside or glucuronide, cleared, and mounted according to the methods described previously.[9,10] Portions of each tissue, or in the younger animals from litter mates, were processed routinely, and paraffin sections were stained with hematoxylin and eosin and by the periodic acid Schiff and Geimsa procedures. Selected incubated sections were lightly counterstained with hematoxylin.

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III. OBSERVATIONS

A. THYMUS

The thymus in the 19-day fetal rat contained reticular cells, lymphocytes, and hematopoietic elements in no definite histologic relationship. By 21 days of gestation, the cell population remained heterogeneous, but a cortical pattern of lymphocytes could be discerned. The newborn and 4-day-old animals had a thin cortical layer composed of predominantly large and medium-sized lymphocytes well demarcated from the medulla. The epithelial cells were pleomorphic in appearance, and occasional rosette formations were present. Macrophages, nucleated red cells, and numerous mitotic figures were seen. By the 7th day, the cortex and medulla were of equal thickness and the cortical medullary cell types were more nearly homogeneous. Mitosis and macrophages were seen in both regions, and epithelial rosettes were more frequent. During the 10th to 14th day the cortex assumed a more follicular organization, and peripheral sinuses, present from birth, were prominent. The epithelial cells appeared differentiated; approximately one-third of the cell population had vesicular nuclei and, in foci, corpuscle formation was identified. In these areas the squamous character of the cells was pronounced, and basophilic stippling was seen in the poorly defined hyaline cytoplasm. Mitotic figures were less common in both layers. By the 28th day thymic corpuscles were numerous. Otherwise, the histologic appearance was similar to that of the 14-day-old animal and the adult. No germinal centers were seen in the sections, and throughout development, the capsule was composed of a delicate reticulum containing lymphocytes and reticular cells (Fig. 1).

GAL-reactive cells were first seen in the 21-day fetal and newborn thymic specimens. The number of positive cells was decreased at 4 and 10 days and only trace reactions were found at 14 days. Numerous intensely staining cells were seen in the 28-day and adult specimens. The changes in GLCR staining were more extreme but similar to those for GAL: moderate activity was seen in the fetal and newborn rats, only trace reactions were present after the 1st week of life, but intense staining was found in the thymus of 28-day-old and adult animals (Fig. 2). The morphologic characteristics of the reactive cells could not be determined with certainty. They appeared to be large lymphocytes, otherwise indistinguishable from surrounding cells and present in both the cortical and medullary regions. Macrophages appeared to be unreactive. Occasional GAL staining in endothelial cells was noted.

FIGURE 1. Newborn (A), 7-Day (C), 14-Day (B), and Adult (D) Thymus. A variably thin cortical layer of lymphocytes was present at birth, but by 7 days the proportion of cortex and medulla was nearly equal. In the older animals, minor changes included a somewhat more follicular appearance of the cortex and greater pleomorphism in the epithelial cell population. Hematoxylin and eosin, 100X.

FIGURE 2. Galactosidase, Adult Thymus. Occasional enzyme staining was found during the 1st week of life. Reactive cells were found with greater frequency, and more intensive staining increased thereafter in both the cortex and medulla. Approximate adult levels of galactosidase and glucuronidase reactivity were found by the 21st day. Uncounterstained section, 125X.

B. SPLEEN

The spleen of the late fetal and newborn rat was a vascular organ with marked hematopoiesis but little or no structural organization. In the 4-day-old specimen, lymphoid aggregates surrounded the small penicillary arteries, and by the 7th day follicles were more compact and a marginal zone was apparent. From the 10th to 14th days the marginal zone increased in size, and numerous mitoses were seen in this area. Erythropoiesis was decreased, but lymphoblasts and megakaryocytes remained prominent. At 28 days the follicles were larger and the marginal zone was relatively thinner. The spleen of the adult animal was similar to that at 28 days except for the presence of germinal centers (Fig. 3).

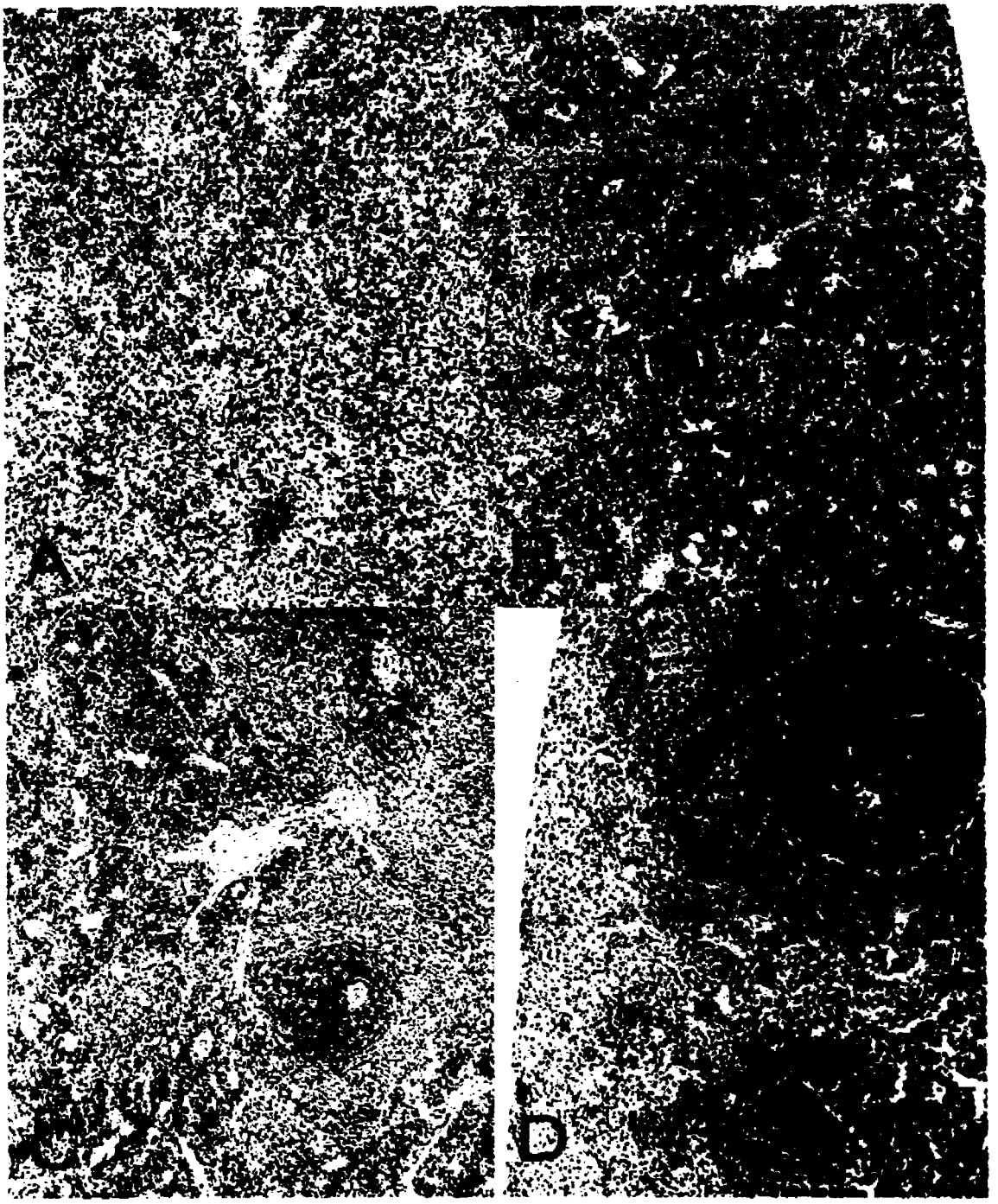

FIGURE 3. Newborn (A), 7-Day (C), 14-Day (B), and Adult (D) Spleen. The newborn rat spleen showed that active hematopoiesis and architectural organization progressed rapidly with compact follicles, a well-developed marginal zone, and germinal follicles. H and E, 100X.

Faint or negative GAL reactions were seen in the spleens of the fetal and early neonatal rats. Moderately reactive cells were first found in the 7- and 10-day specimens. By the 14th day, intensely staining, large mononuclear cells were present in the red pulp adjacent to the follicular margin. This histologic pattern persisted in the 28-day-old and adult rat, although some "background" staining resulted from lesser activity in many of the remaining reticular cells in the red pulp.

The changes with GLCR were again similar, but more extreme. Reactive cells were first seen at 4 days, and they increased progressively in both number and staining intensity in the 7-, 10-, and 14-day specimens. In the latter, prominent background staining also resulted from variable degrees of reactivity in the majority of cells in the red pulp.

The GAL and GLCR staining was localized in the large reticular cells lining the splenic sinusoids. Positive cells were found surrounding the marginal zone and distributed at random in the red pulp. The follicles and marginal zone were uniformly unreactive, but in the adult, a faint ring of staining was seen at the outer rim of the follicle (Fig. 4).

C. LIVER

Kupffer cell development and differentiation in the neonatal liver could not be clearly distinguished from the sinusoidal hematopoietic cells. Hepatocytes showed moderately strong GLCR and less intense GAL reactions at all ages, particularly during the 1st week of life. However, changes in Kupffer cell reactivity could not be estimated reliably.

D. JEJUNUM AND ILEUM

Mononuclear cells, occasionally present in the lamina propria of the fetal jejunum and ileum, increased progressively in number during the 1st month of life. In the younger specimens it was difficult to distinguish fibroblasts and lymphoid elements from the more numerous endothelial cells, but by 2 weeks of age interstitial reticular cells were identified. Infrequent, small mononuclear cell aggregates were first seen on the 4th day; they were both more common and larger by 10 to 14 days. The 28-day specimens contained germinal centers and in every respect resembled the adult tissue.

GAL activity in reticular cells was present in all the specimens and the number of reactive cells increased progressively with age. The increase appeared to parallel that in the total number of interstitial mononuclear cells. The most intense staining in the jejunum was found in the newborn and 4-day rats, but strong reactions were also seen in the remaining specimens. The results were similar in the sections of ileum although the strongest reactions were at 7, 10, and 14 days. GLCR reactions showed only minor differences: fetal and neonatal tissues were negative or only weakly reactive, and the maximum activities were present

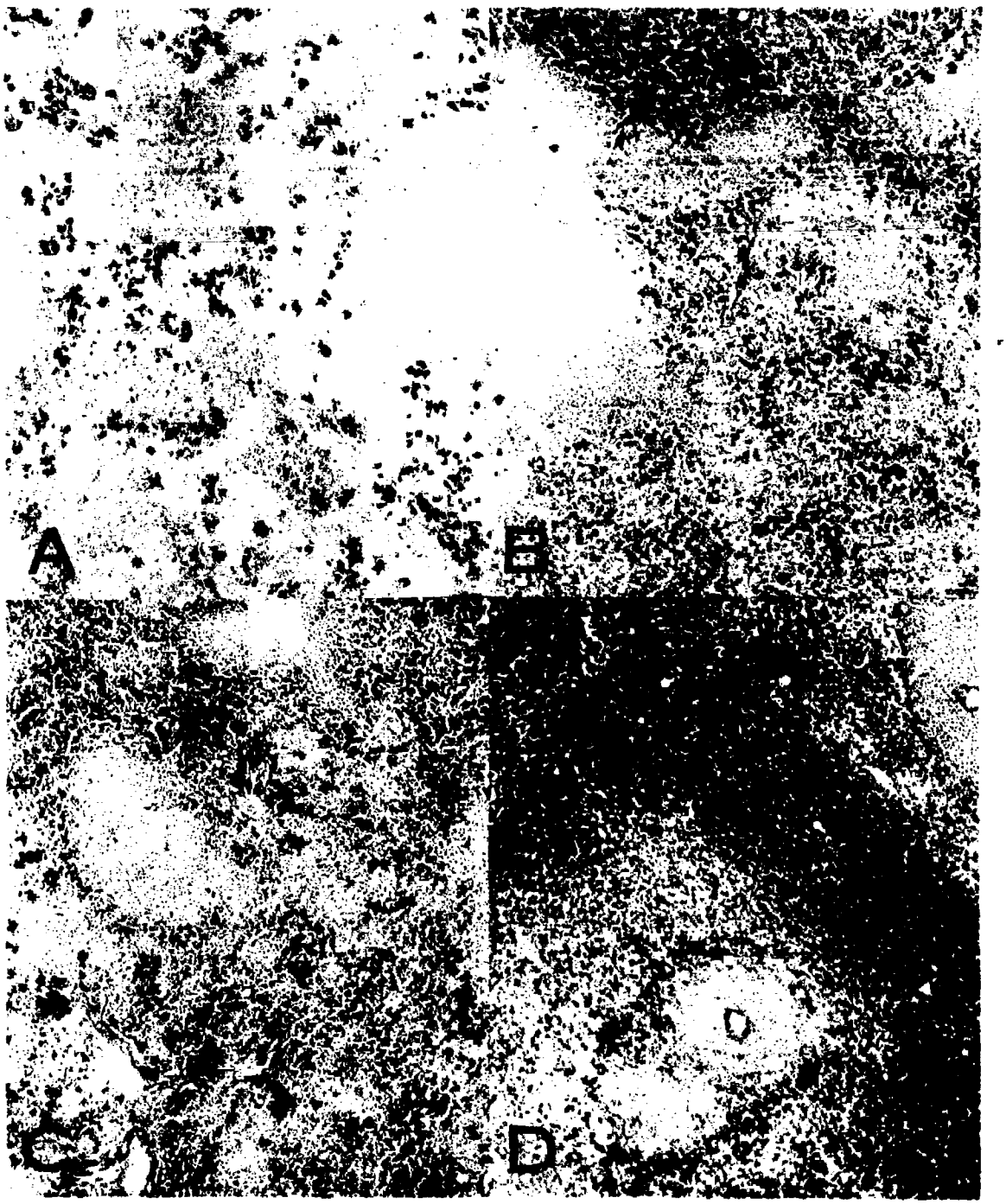

FIGURE 4. Galactosidase, 14-Day (A) and Adult (C) Spleen; Glucuronidase, 14-Day (B) and Adult (D) Spleen. At 2 weeks and in the adult, numerous intensely galactosidase-reactive cells were found throughout the red pulp. Enzymatic activity was uncommon in the marginal zone and absent in the follicle. Histochemical staining was weak during the 1st week of life, and the changes after 14 days were primarily in more discrete localization and background staining. The reactions for glucuronidase in 14-day and in the adult were similar but more marked. Uncounterstained sections, 125X.

in the jejunum and ileum at the same ages. The maturation of Peyer's patches appeared to be the same as that in mesenteric lymph nodes. In several animals, their development was more rapid, but this pattern was not consistent. No correlation with histochemical enzyme reactivity could be made, however, because Peyer's patches were often absent from the segments of intestine used for frozen sections.

E. LYMPH NODES

Mesenteric, axillary, and popliteal lymph nodes in the newborn rat were composed of cellular vascular tissue and small aggregates of reticular cells. The nodes were larger, and so were found with greater ease and frequency, in the 1- and 2-week-old animals. By this time (10 to 14 days), the mesenteric nodes contained identifiable medullary cords and diffuse cortical nodules composed primarily of lymphocytes. By 28 days the hilar tissue was well developed, there were germinal centers, and the architecture closely resembled that in the adult. The same changes were seen in the axillary and popliteal lymph nodes, but the development was more variable, both among the nodes in a single animal and between animals of the same age. Medullary cords and cortical nodules were usually present by 28 days, but germinal centers were not common until 5 or 6 months of age (Fig. 5).

The reactions for GAL and GLCR were also more varied than with the other tissues. Faint reactions were found in some of the newborn specimens, and in general, the intensity of staining and number of reactive cells increased progressively with age. As in the other tissues, a pattern of staining was apparent. Reactive mononuclear cells were located in the peripheral sinuses and medullary cords, but only rarely in or around the cortical nodules (Fig. 6).

F. LUNG

Reticular cells and occasional lymphocytes were present in the submucosal tissue of major bronchi in the first 2 weeks of life. Diffuse aggregates of mononuclear cells were found in four of the six animals at 28 days of age, but a well-developed lymph node architecture was seen only in the 6-month-old rats. Interstitial lymphoid infiltrates were not found in any of the animals. The cryostat sections of lung did not include large bronchi in all cases. In the younger animals there was no peribronchial staining, and reactive cells were infrequent even in the adult animals.

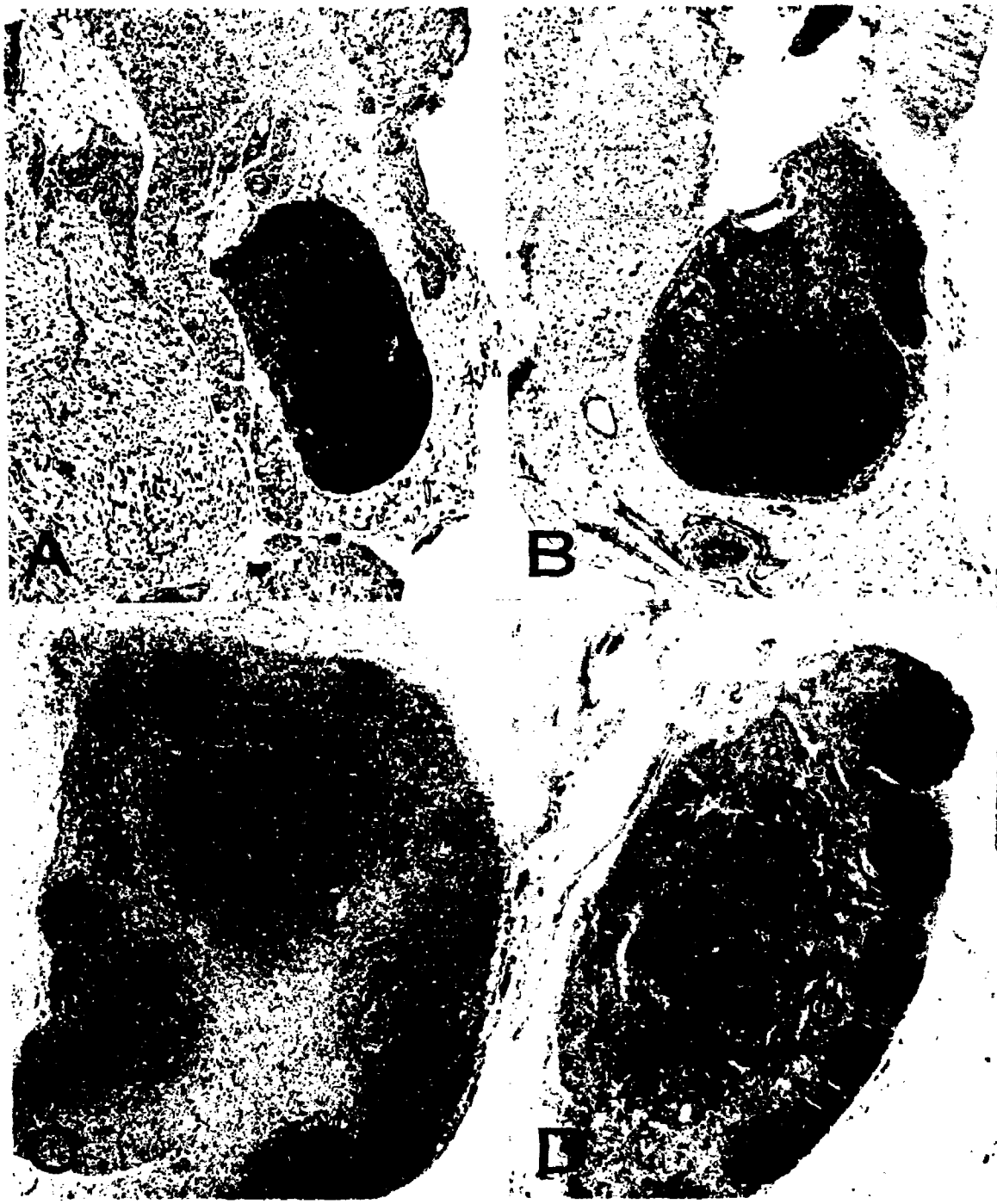

FIGURE 5. Four-Day (A), 14-Day (B), 28-Day (C), and Adult (D) Axillary Lymph Node. During the 1st week of life few organized foci of lymphoid tissue were found draining the extremities but, when present, the mononuclear cells appeared homogeneous and endothelial cells were prominent. By 2 to 4 weeks of age, medullary cords and peripheral sinuses could be identified, but germinal centers were not usually found until age 6 months. H and E. (A), 115X; (B), (C), (D), 65X.

FIGURE 6. Glucuronidase, 4-Day (A) and Adult (B) Axillary Lymph Node. Reactive cells were present at birth and they increased steadily in number and, to a lesser extent, in intensity with age. Localization was not apparent in the neonatal specimens, but in the lymph nodes from adults, cells with enzyme activity were found in the medullary areas but rarely in cortical nodules. The galactosidase reactions were similar in localization and in changes with development. Uncounterstained sections, 115X.

IV. DISCUSSION

The present observations on the morphologic development of the RES in the rat are similar to the pattern of central to peripheral lymphoid maturation that has been described in the rat[11] and in man.[12] Using histologic features that are distinguishing in the adult structure, the sequence of maturation indicates that thymic development is relatively complete at 1 week of life, followed by the spleen and intestinal lymphoid cells, and mesenteric lymph nodes. Finally, and with greater variation, peripheral lymph nodes may not have germinal centers until 6 months of age.

The histochemical studies demonstrated changes in reactivity that were reproducible with respect to both staining intensity and localization in the individual tissues of each age group. Because of the stability and insolubility of the indigoid reaction product, the histochemical preparations were cleared and permanently mounted like paraffin sections, and several dyes were used as counterstains. Nevertheless, in most instances, it was not possible to identify precisely the reactive mononuclear cells. A part of the difficulty was due to the limitations of high-power microscopy on even the most ideal frozen sections. A more significant problem, however, was the morphologic homogeneity of the mononuclear reticular cell population in fetal and neonatal animals. In the majority of slides, the cytologic characteristics of reactive mononuclear cells were in no way different from those of the identical but unstained surrounding cells, and examination of the corresponding paraffin section revealed no cytologic features that might be helpful in distinguishing the cells with enzyme activity.

Another limitation in the interpretation of the histochemical data is the probability that the techniques can detect both lysosomal and nonlysosomal species of the same enzyme. This has been a problem with methods for acid phosphatase[13] and was noted in a previous study of intestinal galactosidase.[14] In the latter, pH differences in staining confirmed the difference in localization between reactive cells in the epithelium and lamina propria. The identical techniques were used in the present study, and it is unlikely that there is a significant nonlysosomal component to the GAL and GLCR reactions.

In spite of the limitations and the absence of quantitation, it is apparent that changes in the reactions for these hydrolytic enzymes in the neonatal RES can, in general, be related to the degree of morphologic differentiation in each tissue. The age at which GAL and GLCR activities approximated the level found in the adult can be compared with the age of morphologic maturation (Table 1). Although each of these ages is somewhat arbitrary, the correlation for the thymus and mononuclear cells in the intestine is not as close as in the other tissues. This poor correlation and the particular location and function of the two tissues suggest a hypothesis to modify the general relationship between morphologic and enzymatic development. The retardation of adult levels of reactivity for

lysosomal enzymes in the thymus may reflect its isolation from antigenic stimulation. Likewise, these enzymes may develop rapidly in the intestinal reticular cells because of the proximity and intensity of antigen in the normal neonatal intestinal tract.

TABLE 1. APPROXIMATE AGES OF MORPHOLOGIC MATURITY AND ADULT LEVELS OF ENZYME REACTIVITY FOR COMPONENTS OF RAT RETICULOENDOTHELIAL SYSTEM

Tissue	Morphologic Maturity	GAL and GLCR
Thymus	7 days	21 days
Spleen	14 days	14 days
Intestine	14 days	10 days
Mesenteric lymph nodes	28 days	1 to 2 months
Peripheral lymph nodes	1 to 6 months	1 to 6 months

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13. ABSTRACT

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14. Key Words

Galactosidase	Enzymes
Glucuronidase	Histopathology
Reticuloendothelial system	Morphology
Histochemistry	Lymphoid tissues

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